APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1992, p. 3660–3666
0099-2240/92/113660-07$02.00/0

Vol. 58, No. 11

An Uncooked Vegan Diet Shifts the Profile of Human Fecal Microflora: Computerized Analysis of Direct Stool Sample Gas-Liquid Chromatography Profiles of Bacterial Cellular Fatty Acids

R. PELTONEN,[1]* W.-H. LING,[2] O. HÄNNINEN,[2] AND E. EEROLA[1]

Department of Medical Microbiology, Turku University, Kiinamyllynkatu 13, 2052 Turku,[1] and Department of Physiology, University of Kuopio, 70211 Kuopio,[2] Finland

Received 24 June 1992/Accepted 2 September 1992

The effect of an uncooked extreme vegan diet on fecal microflora was studied by direct stool sample gas-liquid chromatography (GLC) of bacterial cellular fatty acids and by quantitative bacterial culture by using classical microbiological techniques of isolation, identification, and enumeration of different bacterial species. Eighteen volunteers were divided randomly into two groups. The test group received an uncooked vegan diet for 1 month and a conventional diet of mixed Western type for the other month of the study. The control group consumed a conventional diet throughout the study period. Stool samples were collected. Bacterial cellular fatty acids were extracted directly from the stool samples and measured by GLC. Computerized analysis of the resulting fatty acid profiles was performed. Such a profile represents all bacterial cellular fatty acids in a sample and thus reflects its microflora and can be used to detect changes, differences, or similarities of bacterial flora between individual samples or sample groups. GLC profiles changed significantly in the test group after the induction and discontinuation of the vegan diet but not in the control group at any time, whereas quantitative bacterial culture did not detect any significant change in fecal bacteriology in either of the groups. The results suggest that an uncooked extreme vegan diet alters the fecal bacterial flora significantly when it is measured by direct stool sample GLC of bacterial fatty acids.

The human intestinal microflora is a very complex ecosystem with great metabolic activity, consisting of 1.5 kg of bacterial mass, 10^{11} to 10^{12} individual bacteria per g of bacterial mass, and over 400 different species (6, 4, 15). This microflora exhibits many physiological functions and, in addition, is likely to be involved in different pathological processes (25–27, 29, 31).

Traditionally, there are two major approaches to study the intestinal bacterial flora. The first is the classical bacteriological isolation, identification, and enumeration of different bacterial species. This method is laborious and insensitive, and its reproducibility is poor. Measuring different biochemical components, hormone metabolites, and the mutagenicity and enzyme activities of stool samples is the other approach (8, 22). It is considerably more sensitive, although not necessarily less laborious. This methodology is also more relevant than bacterial taxonomy to the understanding of the intestinal ecology and health impact of the gut flora. Even small dietary changes have caused marked alterations in these parameters, while success in detecting bacteriological changes by using the culture method has been unremarkable (9, 10, 14, 15, 27).

Among many factors normally affecting intestinal bacteriology are nutrients, gut peristalsis, gastric acidity, immunological mechanisms, and intense competition between different bacterial species. Diet is a major factor in determining available nutrients for bacteria, although a part of the nutrients is always from the host. Epidemiological and experimental evidence suggests that diet is an important factor in human illness and also in carcinogenesis. Vegetarian and vegan diets seem to give some protection against certain chronic diseases, including some forms of cancer. One possible mechanism could be the effects of the diet on the intestinal bacteria and their metabolites (2, 24, 32). The purpose of this study was to observe the effect of an extreme uncooked vegan diet on fecal bacteriology by using classical quantitative bacterial culture and automated computerized analysis of gas-liquid chromatography (GLC) bacterial cellular fatty-acid profiles of stool samples. In this method, all of the cellular fatty acids from all of the bacteria, viable or not, present in the stool sample are measured, and all of them contribute to the resulting GLC fatty acid profile according to their relative amounts in the sample. Each individual peak in the profile represents the relative amount of one individual fatty acid. The method is based on the assumption that similar bacterial compositions yield similar fatty acid profiles, and differences as well as similarities can be quantified by the extent to which the profiles resemble each other.

MATERIALS AND METHODS

Participants. Eighteen volunteers, either healthy individuals or patients because of unrelated conditions, were admitted into the study on their informed consent and then divided randomly into two groups. The approval of the Ethical Committee of the University of Kuopio was obtained. Clinical examinations and interviews were performed, lectures on basic nutrition were given, and the purpose of the experiment was carefully explained to the participants at the beginning of the study. Strict adherence to the protocol was continuously stressed throughout the study. No antimicrobial medication was used, and the intake of other medical drugs was reported. Both groups excluded coffee, tea, and

* Corresponding author.

TABLE 1. Anthropometric data and main health problems of the participants

Group	Gender[a]	Age (yr)	Height (cm)	Weight (kg)	BMI[b] (kg/m^2)	Health problems
Test group (living food diet)	F	61	154	68.9	29	Coronary heart disease
	F	65	146	57.2	27	
	F	31	162	74.0	28	
	F	23	167	73.5	27	
	F	56	160	81.4	32	
	M	51	169	73.8	26	Neurosis
	F	50	150	55.1	24	
	M	39	173	63.8	21	
	F	56	162	82.6	31	
Control group (ordinary mixed Western diet)	M	22	191	78.0	21	
	F	51	168	56.9	21	
	F	60	159	70.5	28	Coronary heart disease
	F	35	157	48.7	29	
	M	21	184	76.8	23	Psoriasis with arthritis
	F	41	164	93.6	35	Benign hepatic tumor
	F	19	167	67.5	24	
	F	69	149	62.1	28	Nontoxic goiter
	F	20	170	70.5	24	

[a] F, female; M, male.
[b] BMI, body mass index (weight/height^2).

alcohol from their diet and did not smoke. The anthropometric data of the participants are given in Table 1.

Experimental design. During the first week (days −7 to −1), all of the participants consumed their ordinary diets. At day 0, the test group subjects changed their diet to an uncooked extreme vegan diet for 1 month (intervention period, days 0 to 33), after which they returned to their ordinary diets for the second month (days 34 to 61). The control group stayed on their ordinary diets throughout the study period.

Diets. The uncooked extreme vegan diet used in this study is identified by its advocates as "living food." There are strict rules how to prepare and consume it (30). In the preparation of the test diet, these rules were conscientiously followed. The diet excludes all animal and dairy products. Heating of food is not allowed. A typical feature of the diet is the frequent use of fermentation and mechanical homogenization of some items. This makes it different from so-called raw vegetable food. The average daily diet in the test group during the intervention period was as follows: mung bean sprouts, 79 g; lentil sprouts, 97 g; sprouts, 52 g; buckwheat-beetroot cutlets, 20 g; mixture made of cauliflower, cashew nuts, honey, curry, and tamari seaweed, 20 g; soup made of zucchini, apple, and avocado, 31 g; stew made of buckwheat, carrot, fermented cucumber, and red cabbage, 50 g; marinated mushrooms, 16 g; seaweed rolls, 24 g; buckwheat cutlets, 17 g; almond-curry sauce, 25 g; soup made of zucchini, apple, avocado, and water, 30 g; soup made of cabbage, avocado, and zucchini, 230 g; sunflower seed-garlic-tamari sauce, 80 g; cashew nut-zucchini sauce, 46 g; carrot-nut salad, 47 g; sauerkraut, 100 g; beetroot-carrot-apple cutlets, 27 g; stew made of germinated wheat, carrots, and tamari, 30 g; yogurt made of fermented oats, 216 g; mixture of buckwheat, millet, and figs, 210 g; mixture of sesame seeds, strawberry, and banana, 203 g; strawberry-banana-blueberry sauce, 48 g; carrot juice, 212 g; wheat sprout juice, 100 g; fermented drink made of sprouted wheat, rye, and water, 1,000 g; bread made of sprouted wheat, apple, and water, 12 g; butter made of almonds and water of fermented cucumbers, 30 g.

The control group was advised to continue their ordinary omnivorous diets of mixed Western type.

Diet records. All participants kept daily food records. The content of the living food diet was chemically analyzed. The content of the omnivorous diet was calculated by using 2-day diet records and UNIDAP (Unilever Dietary Analysis Program, PAASIVAARA Ltd., Helsinki, Finland), a computerized diet analysis program using Finnish analytical data. The dietary data are provided in Table 2.

Sample collection. Two baseline samples were collected from each participant during the week before the test period. Only the samples collected at days −2 and −1 were accepted as baseline samples for the test group, whereas in the control group some baseline samples were collected at day 0. After that, the samples were collected at days 5, 13, 19, 26, 33, 40, 47, and 61. They were placed in clear plastic vials at −20°C within 10 min after defecation. In the microbiological laboratory, they were stored at −40°C until analysis.

Direct GLC of the stool samples. GLC was used to produce bacterial cellular fatty acid profiles of the stool samples. For that purpose, the bacterial material first was separated from other mainly fibrous components and free fatty acids of the fecal material as follows: 100 mg of the fecal sample was weighed, suspended in 5 ml of physiological saline, gently mixed, and allowed to remain suspended for 2 h at +4°C. After this, the sample was remixed and allowed to sediment for 15 min, and the bacterial component in the supernatant was removed and centrifuged at 1,000 × g for 15 min at room temperature to produce a pellet.

GLC of the bacterial cellular fatty acids was performed as described previously (3, 16). The collected bacterial mass was saponified, methylated, and analyzed as described previously (3, 16). In brief, the collected bacteria were incubated for 30 min at 100°C in 15% (wt/vol) NAOH in 50% aqueous methanol and then acidified to pH 2 with 6 N aqueous HCl in CH_3OH. The methylated fatty acids were then extracted with ethyl ether and hexane. The GLC analysis was performed with an HP5890A gas chromatograph (Hewlett-Packard) and an Ultra 2,004-11-09B fused silica capillary column (0.2 mm by 25 m; cross-linked 5%

TABLE 2. Nutrient intake from vegan and conventional diets

Nutrient	Intake (amt/day)					
	Test group			Control group		
	Pre[a]	Test[b]	Post[c]	Pre[a]	Test[b]	Post[c]
Energy (kJ)	7,462	7,954	7,547	7,701	7,739	7,051
Fiber (g)	28	45	25	23	23	22
Protein (g)	67	71	62	68	64	55
Carbohydrate (g)	238	276	244	198	216	204
Fat (g)	63	63	59	84	78	68
Fatty acids						
Saturated (g)	24	10	21	33	33	32
Monosaturated (g)	24	31	23	26	28	21
Polyunsaturated (g)	16	20	15	24	16	15
P/S ratio[d]	0.67	2.0	0.65	0.73	0.48	0.46
Vitamins						
Vitamin C (mg)	123.51	<15.25[e]	162.30	135.24	112.24	126.66
Vitamin E (mg)	10.58	11.06	8.20	10.84	10.47	7.83
Thiamine (mg)	1.76	1.73	1.34	1.40	1.08	1.03
Pyridoxine (mg)	1.84	2.57	1.51	1.68	1.81	1.47
Minerals						
NA (g)	2.56	1.22	2.64	2.28	2.74	2.50
K (g)	3.15	5.04	3.37	3.37	3.10	3.10
Ca (g)	0.81	0.52	0.78	1.09	1.06	0.82
Mg (g)	0.38	0.76	0.34	0.41	0.32	0.32
Fe (mg)	16.76	23.5	15.31	14.61	12.96	12.58
Zn (mg)	10.78	16.24	9.09	11.89	9.76	9.10

[a] Pretest period (1 week). The data have been calculated from 2-day diet records pretest in both test and control groups.
[b] Test period (1 month). The test group consumes the living food diet.
[c] Posttest period (1 month).
[d] Polyunsaturated/saturated fatty acids ratio.
[e] Homogenization of the total mass has probably caused a destruction of L-ascorbic acid.

phenylmethyl silicone; Hewlett-Packard). Ultra-high-purity helium was used as the carrier gas. The GLC settings were the following: injection port temperature, 250°C; detector temperature, 300°C; initial column temperature, 170°C, increasing at 5°C/min up to 270°C at 20 min; total analysis time, 25 min; sample volume, 1 μl. The peak retention time and peak area volumes were recorded by an HP3392A integrator (Hewlett-Packard).

GLC data analysis. An application of a bacterial identification program developed previously (3, 11, 21) was used to analyze the GLC profiles of the clinical samples which contained several bacterial species. The analysis of the mixed-flora samples is based on the correlation-and-cluster analysis of the fatty acid spectra of individual samples. This type of analysis can be used to gather the material into clusters containing samples with similar bacterial floras. All of the peaks of individual fatty acids in the chromatograms, i.e., both identified and unidentified peaks, were used in the analyses. The computer analyses were done as previously described (3, 11). All of the samples were compared with each other, and similarity indices were calculated for each sample pair as previously described (3). Similarity indices were presented as correlation matrices and further analyzed by using weighted pair-group cluster analysis of an arithmetic averages method. In addition, the samples were divided into groups by diets and sample days. The resulting groups were compared with each other by calculating the mean differences and standard deviations (SDs) of the GLC profile correlation analyses. When two groups were compared, their difference was determined by calculating the mean value ± SD of all paired similarity index values between the samples in both groups. To get the variation within the groups, these values were calculated as a mean ± SD between all sample pairs within the group. In this way, all of the groups were compared with each other to determine the difference between each group at each time point.

In Fig. 1, some examples of stool sample bacterial fatty acid profiles, comparisons of them with the others, and the resulting similarity indices are presented. In Fig. 1A, a pretest sample (day −2) of a control group individual is compared with the test period sample (day 33) and with a posttest sample (day 47) of the same individual, and in Fig. 1B, the same comparisons between the samples of a test group individual are presented. The height of each individual peak represents the relative amount of one fatty acid in the sample. Figure 1 is shown to demonstrate how computer images of fatty acid profiles of individual stool samples look like and what kind of changes in them can take place as a response to dietary intervention. In these selected examples, very little difference between the samples of a control group individual is observed, whereas a dramatic change can be seen in the test group samples.

Quantitative bacterial cultures. Standardized dilutions were cultured on several aerobic and anaerobic selective culture agars, as detailed in Table 3. Aerobic medias were blood agar and esculin agar for aerobic bacteria and Saboraud agar for yeasts (17). Anaerobic flora was cultured on menadione-cysteine-neomycin agar and on five different selective medias (17, 28). Both aerobic and anaerobic bacteria were cultured for 48 h at 37°C. The anaerobic cultures were done in anaerobic jars. The aerobic bacteria were cultured in a 5% CO_2 atmosphere.

RESULTS

GLC analyses. Figure 2 is a graph of the average difference in GLC profiles of each group on different sample days when

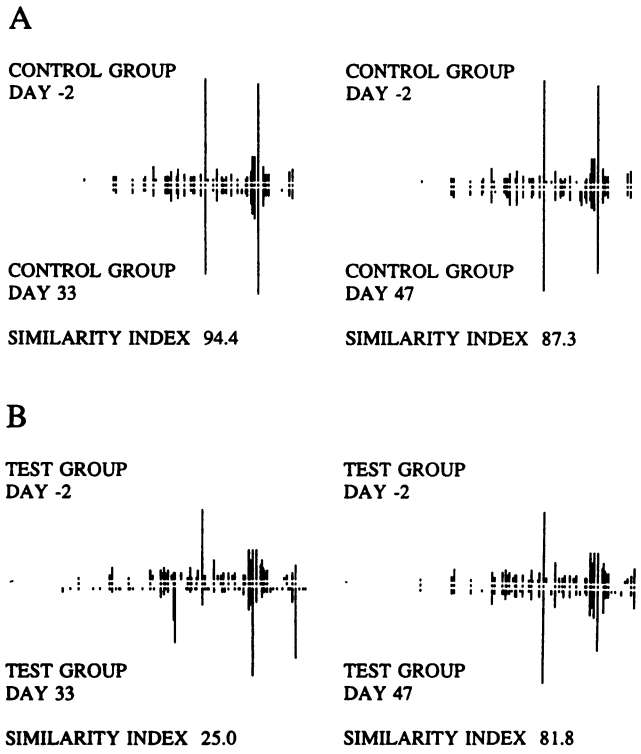

FIG. 1. Four examples of GLC fatty acid profile comparisons. In each of them, two computer images of individual GLC profiles are compared by setting their horizontal baselines over one another. The height of an individual peak represents the relative amount of one fatty acid in the sample. Identical fatty acids are represented in the figures by dots between their horizontal baselines. Calculated similarity indices are presented beside each comparison indicating how much the samples resemble each other. Comparisons are shown between the samples from a control group individual (A) and from a test group individual (B). Day −2 sample is presented above the horizontal baseline in each comparison and is compared with samples from day 33 (left) and day 47 (right).

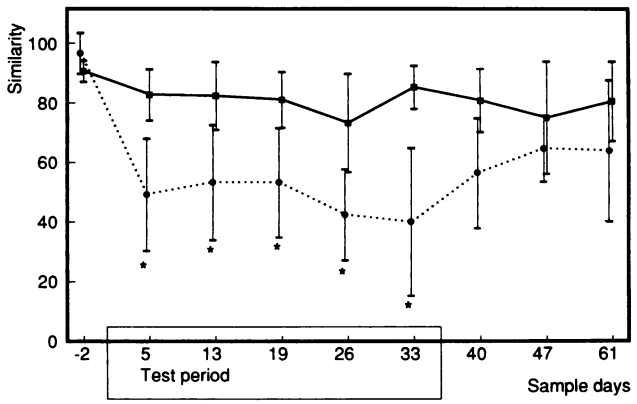

FIG. 2. Effect of the vegan diet on fecal microflora as measured by stool sample GLC fatty acid profile analysis and presented as graphs showing the mean values of the similarity indices ± SDs of all the subjects in control and diet groups, calculated by comparing each sample with the pretest samples of the same individual and counting the arithmetic averages for the groups. The vertical axis shows the average similarity to the pretest samples; the horizontal axis indicates sample days counted from the beginning of the test period. Symbols: ■, control group samples; ●, test group samples; *, statistically significantly ($P < 0.05$) different from the pretest samples (at the same time also statistically different from the control group samples on the same sample day).

compared with their own baseline samples. Each point thus represents the mean value of the similarity indices, calculated separately for every individual of the group between his or her sample on that particular sample day and that from the pretest period. This is the mean change of fecal fatty acid contents from the baseline, and it reflects the change in bacterial floras. Differences between the control and test groups are statistically significant ($P < 0.05$) on days 5, 13, 19, 26, and 33 (test diet period, days 0 to 33) and also within the test group when the test period samples are compared with either the pretest or posttest samples. This indicates that significant changes take place in the test group first after the introduction of the vegan diet and later after the reintroduction of conventional diet (days 40, 47, 61), whereas no such change in the control group is observed at any time.

In Fig. 3, both groups on different sample days are clustered by the extent of their resemblance to each other. Two separate clusters are formed; in the first cluster are all of the samples from both groups while on a conventional diet (except test group day 40) and in the other are the samples from the test group while on the vegan diet. In between these clusters is the sample day 40 of the test group, indicating a gradual return of the GLC fatty acid profiles towards the baseline at that time.

Statistical P values for the differences between the sample

TABLE 3. Media and methods employed for quantitative bacterial culture of stool samples

Media	Dilutions (wt/vol) (0.25 mg of feces × used dilution/plate)	Target microbes
Aerobic flora		
Blood agar	10^{-2}, 10^{-4}	Aerobic bacterial flora
Saboraud agar	10^{-1}, 10^{-3}	Yeasts
Esculin agar	10^{-1}, 10^{-3}, 10^{-5}	Enterococci
Anaerobic flora		
Menadione-cysteine-neomycin agar	10^{-3}, 10^{-5}, 10^{-7}	Anaerobic flora
Menadione-cysteine-neomycin-vancomycin agar	10^{-3}, 10^{-5}, 10^{-7}	Anerobic gram-negative flora
Menadione-cysteine-neomycin-colistin agar	10^{-3}, 10^{-5}, 10^{-7}	Anaerobic gram-positive flora
Bacteroides-bile-esculin agar	10^{-1}, 10^{-3}, 10^{-5}	*Bacteroides fragilis* group
Cycloserine-cefoxitin-fructose agar	10^{-1}, 10^{-3}	*Clostridium difficile*
Rogosa agar	10^{-1}, 10^{-3}, 10^{-5}	Lactobacilli

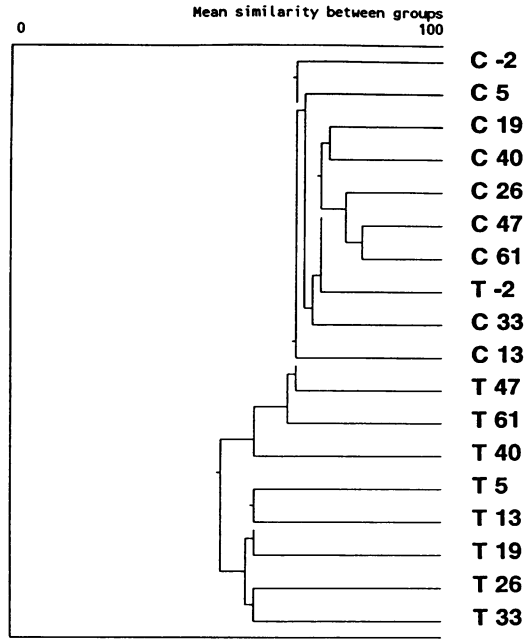

FIG. 3. Dendrogram representing relative similarities of stool sample GLC fatty acid profiles on a scale from 0 to 100 between the samples of the vegan diet group and the samples of the control group taken on different sample days. C, control group; T, test group. Numbers refer to the sample days.

day groups are shown in Fig. 4. An accumulation of statistically significant P values (<0.05) can be observed in the comparisons of the test period samples of the test group with other sample day groups.

Quantitative bacterial culture. The results of quantitative bacterial cultures for the control and the test groups are shown as graphs in Fig. 5. No significant change could be detected in either of the groups during the study period by the culture method.

DISCUSSION

Fermentation is a typical feature used frequently in food preparation in the uncooked extreme vegan diet known as living food. Microorganisms associated with lactic acid fermentation include species found primarily in the following genera: *Streptococcus*, *Lactococcus*, *Pediococcus*, *Leuconostoc*, and *Lactobacillus* (12). The fermented items contain large numbers of lactobacilli. Some of the supposed beneficial effects of the diet has been thought to be due to lactobacilli. Lactobacillus supplements to the diet have, indeed, decreased the fecal bacterial enzyme activities in subjects consuming a mixed Western diet toward the levels found in subjects on a permanent vegan diet (8).

Ling and Hänninen (12a) tested the material used in this study for certain fecal bacterial enzyme activities and were able to show considerable decrease in them during the living food diet. Because the diet contains large numbers of lactobacilli, some increase in fecal lactobacilli counts was expected. However, no significant change in the numbers of lactobacilli or any other bacteria was seen in either group. This may seem surprising, but it is in accordance with several studies on the effects of short-term dietary modifications (4, 14, 15, 27). When populations that are permanently on special diets are tested, some differences in bacterial counts have been detected, with vegetarians usually having higher counts of lactobacilli (5, 7). In our study, the variation in bacterial counts between individuals and between sample days within each group was quite considerable and may partly explain the statistical insignificance. One possible explanation may also be the shortness of the experimental period, i.e., only 1 month. A longer experimental time may be needed so that the changes could be big enough for the culture method to detect them. There is also a possibility that the microflora was markedly altered, as indicated by enzyme activities and GLC, but the changes took place mainly in the upper colon and were not detectable by culturing fecal bacteria.

GLC of bacterial cellular fatty acids has been successfully used as a routine laboratory method in our laboratory for the identification of isolated bacterial species (3). It has also

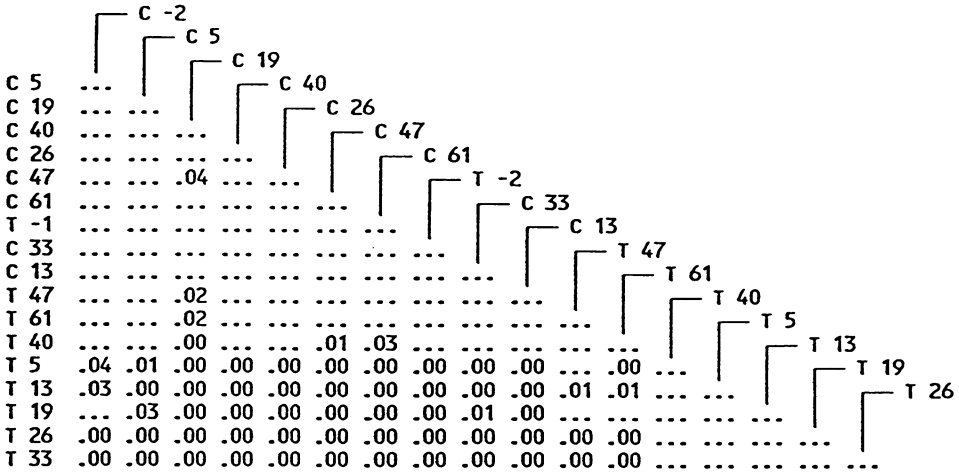

FIG. 4. Statistical P values for the differences in GLC fatty acid profiles between the groups on different sample days, calculated by using student's t test. All of the P values of <0.05 are shown to two decimals. C, control group; T, test group. The numbers after the symbols refer to the sample days.

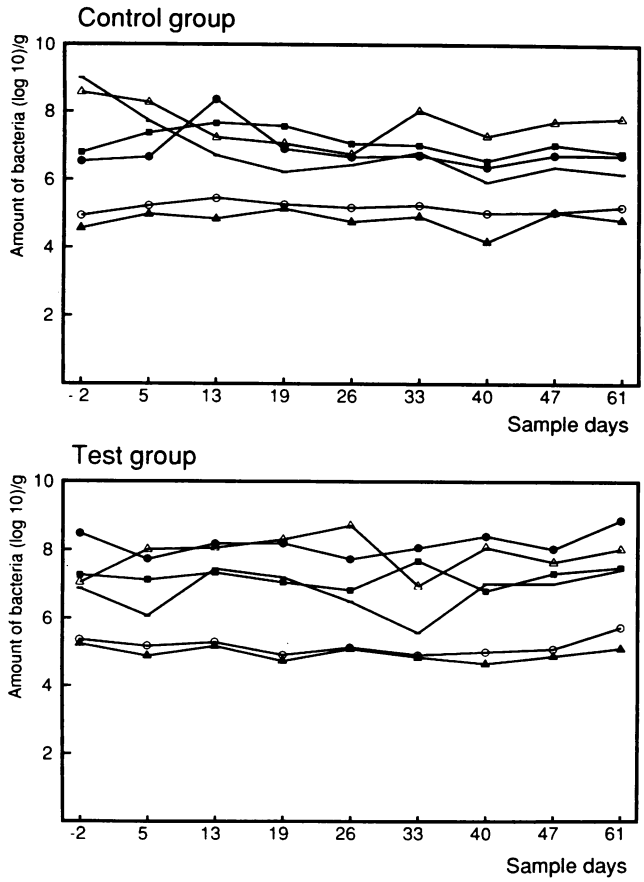

FIG. 5. Amounts of bacteria per gram of feces. The vertical axis shows the mean number of different bacteria as logarithms, and the horizontal axis indicates sample days. Symbols: ■, *Bacteroides* sp.; ●, aerobic gram-negative rods; ▲, anaerobic gram-positive bacteria; ——, *Lactobacillus* sp.; ○, anaerobic gram-negative bacteria; △, *Enterococcus* sp.

been applied to the epidemiological analysis of isolated bacterial strains (11). In this study, the samples for GLC of bacterial cellular fatty acids were stool specimens instead of isolated bacterial species or strains. Peltonen and Eerola have recently demonstrated a direct GLC of stool samples that is capable of detecting gross changes of fecal microfloras induced in rats by antibiotics and very extreme dietary alterations such as an all-meat diet or a dry-fish-powder diet (21). These factors are known to induce bacterial changes of such magnitude that the classical bacteriological techniques have also been able to detect them (1, 18–20, 23). In the present study, GLC was applied in such a human dietary experiment, where the culture method in the past has been unreliable (4, 6, 9, 10, 13). A computerized comparison of the fatty acid profiles produced by direct GLC of the stool samples was able to detect statistically significant alterations in the fatty acid contents of the test group samples in response to the introduction as well as the discontinuation of the vegan diet. This is in accordance with the results of bacterial enzyme activity changes. The GLC method indicates that there is an actual alteration of bacterial flora causing the enzymatic changes.

GLC is especially suitable for the studies of multibacterial specimens such as fecal samples. It is fast, technically quite simple to perform, and especially useful when the number of samples is large. The main disadvantage of the method is that it does not give taxonomic names for the bacteria if the sample contains several different species. However, we are enhancing the ability of the method to identify bacterial species in the samples containing several species by automatically comparing the fatty acid profile of such a sample to the known profiles of different bacterial species and using in the comparison only the fatty acids present in the particular species with which the sample is compared at that time.

We conclude that the dietary change to the uncooked extreme vegan diet known as living food and the return to conventional mixed Western diet both induce statistically significant alterations in fecal microfloras within 1 to 2 weeks as measured by GLC of stool sample bacterial cellular fatty acids, whereas the classical bacteriological techniques were not able to detect any significant microfloral changes. This suggests that the GLC method is not only much faster but also considerably more sensitive than quantitative bacterial culture in detecting changes in fecal microfloras. It is probably the simplest way to get answers to the following questions: Is there change and how big is the change in bacterial flora induced by any environmental factor such as diet, medication, or disease.

ACKNOWLEDGMENTS

We thank Kirsti Tuomela and Marja-Riitta Teräsjärvi for their excellent technical assistance.

This work was supported financially by Juho Vainio Foundation and the Finnish Gastroenterological Society.

REFERENCES

1. **Crowther, J. S., B. S. Drasar, P. Goddard, M. J. Hill, and K. Johnson.** 1973. The effect of a chemically defined diet on the faecal flora and faecal steroid concentration. Gut **14**:790–793.
2. **Dwyer, J. T.** 1988. Health aspects of vegetarian diets. Am. J. Clin. Nutr. **48**:712–738.
3. **Eerola, E., O.-P. Lehtonen.** 1988. Optimal data processing procedure for automatic bacterial identification by gas-liquid chromatography of cellular fatty acids. J. Clin. Microbiol. **26**:1745–1753.
4. **Evaldson, G., A. Heimdahl, L. Kager, and C. E. Nord.** 1982. The normal human anaerobic microflora. Scand. J. Infect. Dis. Suppl. **35**:9–15.
5. **Finegold, S. M., H. R. Attebery, and V. L. Sutter.** 1974. Effect of diet on human fecal flora: comparison of Japanese and American diets. Am. J. Clin. Nutr. **27**:1456–1469.
6. **Finegold, S. M., V. L. Sutter, and G. E. Mathisen.** 1983. Normal and indigenous intestinal flora, p. 3–31. *In* D. J. Hentges (ed.), Human intestinal microflora in health and disease. Academic Press, Inc., New York.
7. **Finegold, S. M., V. L. Sutter, P. T. Sugihara, H. A. Elder, S. M. Lehmann, and R. L. Phillips.** 1977. Fecal microbial flora in Seventh-Day Adventist populations and control subjects. Am. J. Clin. Nutr. **30**:1781–1792.
8. **Goldin, B. R., L. Swenson, J. Dwyer, M. Sexton, and S. L. Gorbach.** 1980. Effect of diet and lactobacillus acidophilus supplements on human fecal bacterial enzymes. J. Natl. Cancer Inst. **64**:255–261.
9. **Hentges, D. J.** 1980. Does diet influence human fecal microflora composition? Nutr. Rev. **38**:329–336.
10. **Hentges, D. J., B. R. Maier, G. C. Burton, M. A. Flynn, and R. J. Tsutakawa.** 1977. Effect of a high-beef diet on the fecal bacterial flora of humans. Cancer Res. **37**:568.
11. **Kotilainen, P., P. Huovinen, and E. Eerola.** 1991. Application of gas-liquid chromatography analysis of cellular fatty acids for species identification and typing of coagulase-negative staphylococci. J. Clin. Microbiol. **29**:315–322.
12. **Lindgren, S. E., and W. J. Dobrogosz.** 1990. Antagonistic

activities of lactic acid bacteria in food and feed fermentations. FEMS Microbiol. Rev. **87:**149–164.

12a. **Ling, W. H., and O. Hänninen.** Shifting from a conventional diet to an uncooked vegan diet reversibly alters fecal hydrolytic activities in humans. J. Nutr., in press.

13. **Maier, B. R., M. A. Flynn, and G. C. Burton.** 1974. Effects of a high-beef diet on bowel flora: a preliminary report. Am. J. Clin. Nutr. **27:**1470–1474.

14. **Moore, W. E. C., E. P. Cato, I. J. Good, and L. V. Holdeman.** 1981. The effect of diet on the human fecal flora, p. 11–24. *In* W. R. Bruce, P. Correa, M. Lipkin, S. R. Tannenbaum, and T. D. Wilkins (ed.), Banbury Report 7. Gastrointestinal cancer: endogenous factors. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

15. **Moore, W. E. C., and L. V. Holdeman.** 1975. Discussion of current bacteriological investigations of the relationships between intestinal flora, diet, and colon cancer. Cancer Res. **35:**3418–3420.

16. **Moss, C. W., and O. L. Nunez-Montiel.** 1982. Analysis of short chain acids from bacteria by gas liquid chromatography with fused silica capillary column. J. Clin. Microbiol. **15:**308–311.

17. **Nash, P., and M. M. Krenz.** 1991. Culture media, p. 1226–1288. *In* A. Balows, W. J. Hausler, K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.

18. **Nord, C. E., A. Heimdahl, and L. Kager.** 1986. Antimicrobial induced alterations of the human oropharyngeal and intestinal microflora. Scand. J. Infect. Dis. Suppl. **49:**64–72.

19. **Nord, C. E., A. Heimdahl, L. Kager, and A. S. Malmborg.** 1984. The impact of different antimicrobial agents on the normal gastrointestinal microflora of humans. 1984. Rev. Infect. Dis. Suppl. **6:**270–275.

20. **Nordenvall, B., D. Hallberg, L. Larsson, and C. E. Nord.** 1983. The effect of clindamycin on the intestinal flora of patients with enteric hyperoxaluria. Scand. J. Gastroenterol. **18:**177–181.

21. **Peltonen, R., and E. Eerola.** 1992. Direct automatic bacterial analysis of rat stool samples; the effects of diet and medical treatment studied by computerized gas-liquid chromatography of bacterial fatty acids. Microb. Ecol. Health Dis. **5:**93–103.

22. **Reddy, B. S., J. H. Weisburger, and E. L. Wynder.** 1974. Fecal bacterial beta-glucuronidase: control by diet. Science **183:**416–417.

23. **Reeves, D. S.** 1986. The effect of quinolone antibacterials on gastrointestinal flora compared with that of other antibacterials. J. Antimicrob. Chemother. Suppl. **18:**89–102.

24. **Rowland, I. R., A. K. Mallet, and A. Wise.** 1985. The effect of diet on the mammalian gut flora and its metabolic activity. Crit. Rev. Toxicol. **16:**31–103.

25. **Savage, D. C.** 1986. Gastrointestinal microflora in mammalian nutrition. Annu. Rev. Nutr. **6:**155–178.

26. **Shinebaum, R., V. C. Neumann, E. M. Cooke, and V. Wright.** 1987. Comparison of faecal florae in patients with rheumatoid arthritis and controls. Br. J. Rheumatol. **26:**329–333.

27. **Simon, G. L., and S. L. Gorbach.** 1984. Intestinal flora in health and disease. Gastroenterology **86:**174–193.

28. **Sutter, V. L., D. M. Citron, M. A. C. Edelstein, and S. M. Finegold.** 1986. Wadsworth anaerobic bacteriology manual, 4th ed. Star Publishing Co., Belmont, Calif.

29. **Wensinck, F., V. L. Custers, K. P. A. Poppelaars, and A. M. Schroder.** 1981. The faecal flora of patients with Crohn's disease. J. Hyg. Lond. **87:**1–12.

30. **Wigmore, A.** 1980. Recipes for longer life. Avery Publishing Group, Inc., Wayne, N.J.

31. **Wilkes, E., and E. S. Meek.** 1979. Rheumatoid arthritis: review of searches for an infectious cause. II. Infection. **7:**192–197.

32. **Ziegler, R. G.** 1991. Vegetables, fruits and carotenoids and the risk of cancer. Am. J. Clin. Nutr. **53:**251–259.